Effect of *Carica papaya* Extract on Seed Borne Fungal Organism of African Yam Bean (*Sphenostylis stenocarpa* Hochst ex. A. Rich. Harms) Seeds

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KEYWORDS

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ABSTRACT

Extracts of many higher plants have been reported to exhibit antifungal properties under Laboratory experiment. This study was therefore carried out to investigate the effect of Carica papaya plant extracts on seed borne pathogen of African yam bean. Seed health test of African yam bean was carried out using blotter paper method. Test of plant extract for inhibition of radial growth of seed borne pathogen of African yam bean was studied under in-vitro experiment at 0%, 50%, 75% and 100% concentrations with 0% as the control. The design used was a completely randomized design (CRD) with three replications. The test plant extracts of different concentrations were added into petri dishes containing molten sterilized Sabouraud Dextrose Agar (SDA) and swayed gently on the Laboratory bench to allow even mixing. These were allowed to gel. Then nine-millimeter discs of a seven-day pure culture of Aspergillus flavus were aseptically placed on the center of the petri dishes containing the SDA-extract mixture. Record on radial inhibition effect of the test plant extract was kept for further analysis. Analysis of variance (ANOVA) was conducted on data on radial inhibition of test fungus. Results showed that Aspergillus niger, Aspergillus flavus and Aspergillus terreus were isolated from incubated African yam bean seeds. Results also showed that Carica papaya leaf extract used was very effective and the higher the concentrations of extract, the more effective in the inhibition of radial growth of the test fungal organism. It could therefore be recommended that farmers should always conduct viability test on procured seeds for planting. That farmers should rather use plant extract such as was used in this investigation if stabilized in controlling seed borne fungal pathogens of African yam bean seeds than synthetic fungicides.

INTRODUCTION

African yam bean (AYB) *Sphenostylis stenocarpa*, belongs to the legume family. It originated in Ethiopia (Busson, 2001), but both wild and cultivated types now occur in tropical Africa as far north as Egypt and also throughout West Africa from Guinea to Southern Africa. It is cultivated in Nigeria mainly for seed and also grown for tubers in Cote d'Ivoire, Ghana, Togo, Cameroon, Gabon and Democratic Republic (Utter, 2007). The African yam bean (*Sphenostylis stenocarpa*) is a climbing legume that grows to a height of over 3m and is adapted to low land tropical conditions. It is one of the lesser-known legumes (Ikhajiagbe *et al.*, 2007a, b; Apata and Ologhobo,1990). Different authors over the years have reported the proximate composition of African yam bean with varying values over the years, Carbohydrate (49.88–63.51%) and protein (19.53–29.53%) are the major components of AYB, while other components such as ash (1.86–5.35%), fat (1.39–7.53%) and fibre (2.47–9.57%) are present in relatively small amounts (Adeyeye *et al.*, 1994). African yam bean (*Sphenostylis stenocarpa*) is a hard-to-cook under exploited leguminous plant grown extensively in Western Africa (Enujiugha *et al*, 2012; Uchegbu, 2015). For efficient growth of this crop there is need for seed viability test. Seed health is a measure of freedom of seeds from pathogens. The presence or absence of seed-borne pathogens can be confirmed through the use of seed health testing (Agrawal,1997). The term "seed health" include the incidence in the seed lot of fungi, bacteria, viruses, and animal pests such as nematodes and insects. The test used depends on the organism being tested for and the purpose of the test quality assurance or phytosanitary purposes when seed is exported (ISTA, 2015). It includes visual examination of seeds externally or internally, macro or microscopically for the presence of pathogens as well as incubating seeds on agar or moist blotter papers and identifying the pathogens microscopically (Warham *et al*.,1990). In seed health testing for seed borne fungi pathogens the blotter test is no doubt one of the most important method available (limonard,1966). Blotter tests are similar to germination test in that seeds are placed on

moistened layers of blotter and incubated under conditions that promote fungal growth. The demand for the biological control of pathogens using plant extracts containing secondary metabolites has been a common practice for thousands of years (Chavez-Quintal *et al*.,2011; Hewajulige, Dhekney, 2016). Papaya (*Carica papayaL*.) belongs to the family Caricaceae and is the most economically important species of the genus *Carica*. (Hewajulige, Dhekney,2016) Papaya is native to tropical America, and seeds of papaya were taken from the Caribbean, to Malacca or Philippines, then to India. Subsequently, papaya was introduced as a plantation crop to Australia, Hawaii, Sri Lanka, and other tropical and subtropical countries in the world (Hewajulige, Dhekmeg, 2016). The papaya plant, including fruit, leaf, seed, bark, latex, and their ingredients play a major role in the management of disease progression. *Carica papaya* leaf contains active components such as alkaloids, glycosides, tannins, saponins, and flavonoids, which are responsible for its medicinal activity.

MATERIALS AND METHODS

Germination test and seed health test: Germination test was carried out to investigate the rates at which the test samples germinate and also seed health test was carried out to investigate the seed borne pathogen of the test sample. The percentage germination of African yam bean seed was determined by this formula:

$$\frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times \frac{100}{1}$$

The percentage infection of African yam bean seed was determined by this formula:

$$\frac{\text{Number of infected seeds}}{\text{Total number of seeds}} \times \frac{100}{1}$$

Seed health test: Seed health test for seed borne fungi was carried out following the rules of international seed testing association (ISTA, 2001). Standard blotter method was used for this study. In the blotter paper method, 12 seeds each was randomly taken from the 204 seeds. The seeds were sterilized with a mixture of 10ml of ethanol and 90ml of water (10% ethanol) for 3 minutes and rinsed two times with sterile distilled water in order to effectively remove surface contamination without affecting the germination percentage of African yam bean seeds (Pernezny *et al*, 2002). Twelve seeds of African yam beans were plated in each 9cm petri-dish containing three layers of Whatman filter paper wetted with distilled sterile water. Seeds were arranged according to international seed testing association (ISTA, 2001). Seventeen petri-dishes were used for the sample. The twelve seeds of African yam bean in a plate were arranged seven seeds at the outer ring, four seeds at the middle ring and 1 seed in the inner ring. More water was added to rewet the paper after initial wetting after some dryness was experienced. The petri-dish were sealed up using masking tape and labeled properly. The plates were placed on the Laboratory working bench which was first sterilized with methylated spirit to ensure an aseptic condition. Germination and infection counts were observed for seven days from the second day after plating. Germination and infection counts were recorded every day until the seventh day. Fungal growth was observed on the plates based on the mycelia colour and hyphal growth.

Culturing of fungal organisms from infected seeds: Seeds infected with fungal organisms were isolated from the plated seeds and transferred into Sabouraud dextrose agar (SDA) medium. The organisms were incubated in triplicate plates at 28°C ±2 for seven days. Sub-culturing of isolated fungal organisms was done using SDA media to obtain pure cultures, which were kept properly for further investigation.



Plate1: Plated seeds of AYB on day1



Plate 2: Germinated seeds



Plate 3: Infected seeds of African yam bean

Microscopic identification of isolated fungal organisms: Temporary slides of isolated fungal organisms were made by placing small portion of the mycelia of each fungus taken from the part active growth on the sterile glass slides having some drops of lactophenol in cotton blue and the slides were covered with a cover slip. The prepared slides were viewed under a compound microscope model (Olympus-XN50). Identification of the fungal organisms was based on the culture growth patterns, colour of

mycelia and microscopic examination of vegetative and reproductive structures. Micrographs of the organisms were also taken and recorded.

***In- vitro* effect of different concentrations of *Carica papaya* plant leaf extract:** Different concentrates of the agar extract mixture were dispensed into 9cm petri-dishes and allowed to gel and then inoculated centrally with 0.9cm diameter mycelial discs obtained from seven- days pure culture of *Aspergillus flavus with* a sterilized cork-borer and placed at the center of each petri-dish. Two perpendicular lines were marked thinly on the base of the petri-dish with a marker and passing through the center of the petri-dish to serve as reference for measuring growth. All plates were placed on a Laboratory bench and at a room temp of 28 ± 2°C. Radial growths along each line were measured at exactly 24hrs interval with a meter rule to determine the radial growth inhibition. The radial growth inhibitions in each plate were measured for 7 days. Each treatment was replicated three times. Percentage radial inhibition was determined according to (Sundar *et al*, 1995) thus: Percentage radial inhibition = $\frac{dc-dt}{dc}$ x $\frac{100}{1}$ Amadioha (2003). Where dc = control,

dt = treatment.

Experimental design/Data analysis: The experiment was laid out in a completely randomized design (CRD) and replicated three times. Data collected were subjected to analysis of variance (ANOVA) and means were separated using least significance difference (LSD) at 5% probability level. GenStat release 10.3 version was used for all the statistical analysis

RESULTS

Germination and infection percentage of plated African yam bean (*Sphenostylis stenocarpa*)

The germination and infection percentages of S*phenostylis stenocarpa* were calculated for seven days. Having seventeen petri-dishes, each having twelve seeds. Germination and infection percentages were calculated for each petri-dish for the period of seven days. The highest germination percentage was recorded as 92% in replicate 4 on the 6th and 7th day, followed by 83% in replicate 7 on the 6th and 7th day. The highest infection percentage was recorded at 50% in replicate 7 on the 6th and 7th day From day 1 till day 3, no germination was seen; therefore, there was 0% germination in the 17 petri-dishes for that period.

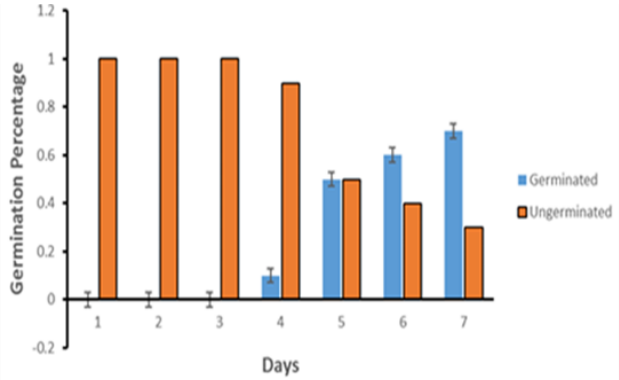

Figure 1: Percentage germination of African Yam Bean for seven (7) days

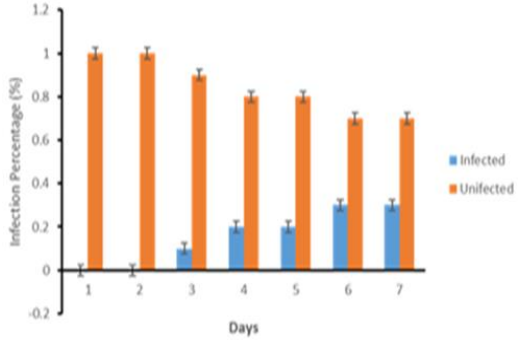

Figure 2: Percentage Infection of African Yam Bean for seven (7) days

Fungal isolation and identification: The result of isolation and identification of fungal seed borne organisms showed that *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus terreus* were implicated.

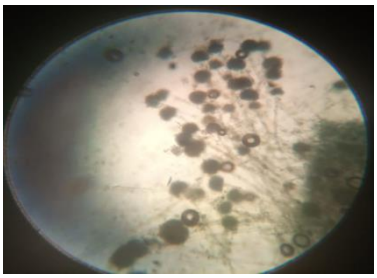

Plate 4: *Aspergillus niger*



Plate 5: Micrograph of *Aspergillus niger*



Plate 6: *Aspergillus flavus*

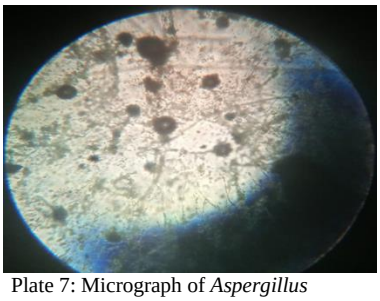

Plate 7: Micrograph of *Aspergillus flavus*



Plate 8: *Aspergillus terreus*

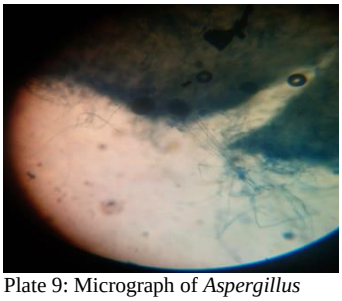

Plate 9: Micrograph of *Aspergillus terreus*

Effect of plant extract, their concentration on the percentage inhibition on the radial growth of *Aspergillus flavus*: Result of phytotoxic effect of *Carica papaya* plant leaf extract showed that there was significant inhibitory effect of the extract on radial growth of *Aspergillus flavus* in culture. Also, the result showed that there was significant difference among the effects of the various concentrations in inhibiting the growth of the test organism in culture. On day two, concentration of 100% had the highest (86.7%) inhibition followed by 75.0% obtained in concentration of 75% while the least (62.3%) inhibition was observed in 50% concentration level. All the three concentrations performed better than the control. This trend was observed in day three till the seventh days

Table 1: Effect of *Carica papaya* plant extracts by Soxhlet extractions, their concentration and percentage inhibition on the growth of *Aspergillus flavus* from day 1 to 7

Concentration and Percentage inhibition of *Aspergillus flavus* in culture							
Con (%)	**Day1**	**Day2**	**Day3**	**Day4**	**Day5**	**Day6**	**Day7**
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
50.00	0.00	62.3	57.67	78.33	78.67	67.70	34.70
75.00	0.00	75.0	73.67	85.00	84.00	82.0	72.00
100.00	0.00	86.7	83.67	90.67	88.00	88.00	88.00
Grand Mean	0.00	56.0	53.75	63.50	62,67	59.40	48.80
$LSD_{0.05}$	0.00	8.78	6.32	3.72	3.95	10.11	18.14

DISCUSSION

The result of the germination test showed that germination of African yam bean is not instant; it takes 3 or 4 days before germination starts. Seed treatment is the safest and the cheapest way of control of seed-borne fungal diseases and to prevent bio-deterioration of grains (Chandler 2005; Bagga and Sharma 2006). The efficiency of four seed health testing techniques namely the blotter, deep-freezing blotter, ragdoll and agar plate methods in detecting seed-borne fungi of African yam bean, *Sphenostylis stenocarpa* seeds was evaluated and the blotter method was found to be the most suitable testing technique for detecting Aspergilus *flavus, A niger, A terreus.* Ora *et al*. (2011) reported that the seed health testing in seed companies as well as those working in Research and quarantine departments becomes about 90% of the food crops grown world-wide and the plant germplasm being distributed between and within countries are propagated by true seeds (Neergaard, 1979). Many plants pathogen (bacteria, fungi, nematodes and viruses) affecting the food crop and plant germplasm are seed borne and seed transmitted.

Isolation of seedborne fungal organisms: The result on isolation and identification of seedborne fungal organisms from *Sphenostylis sternocarpa* showed that *Aspergillus* species were implicated which included: *Aspergillus niger, Aspergillus flavus* and *Aspergillus teureus.* This is similar to results of Iwuagwu *et. al* (2019) and Iwuagwu *et al* (2022), who isolated *Aspergillus niger* from seeds of **Vigna subterranean** and *Citrullus lanatus* respectively

Phytotoxic effect of *Carica papaya* plant leaf extract on radial growth of *Aspergillus*

Flavus: The result of the effects of C*arica papaya* leaf extract on inhibition of radial growth of *Aspergillus flavus* isolated from *Sphenostylis sternocarpa* showed that 50% had the lowest fungal radial growth inhibition compared to C*arica papaya* leaf extract concentration of 75% and 100%. This corroborates Olubode *et al* (2018), who reported exhibition of antifungal properties of *Carica papaya* leaf extract against *Aspergillus niger* and *Aspergillus flavus* under laboratory trails. This is also in line with works of Aliero and Afolayan (2006), who also reported antimicrobial activity of *Solanum tomentosum.* Parekh *et al.* (2006) and Iwuagwu *et al* (2022) have as well reported about efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity against seedborne pathogenic fungi

CONCLUSION

From the experiment it was observed that all organisms isolated were *Aspergillus species* Therefore, it could be said that fungal organism were the major cause of seeds deterioration of African yam bean, It could also be inferred that seeds deterioration caused by *Aspergillus spp*, leads to poor visibility and loss of seedling vigour. The *Carica papaya* extract used was very effective in inhibiting *Aspergillus flavus* in culture. It was also observed that the higher the concentrations of the extract, the more the effectiveness in inhibition of radial growth of the fungal organism

RECOMMENDATIONS

Having seen that African yam bean losses viability when infected, it could therefore be recommended that farmers should always source their seeds from certified seed outlets. Also, farmers should always conduct viability test before sowing to ensure adequate plant establishment in the field. Farmers should rather than using synthetic protective chemicals which are detrimental to human health and environment use plant extracts such as was tested in this research to control fungal diseases, which are also readily available.

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